Methods: Experimental OA was induced by intra-articular injection of MIA into right hind knee of male Wistar rats. Mechanical hyperalgesia and knee joint pain were measured by von Frey filament and Incapacitance Tester, respectively. First, we examined the dose- and the time-dependency of analgesic effect of single administration of Neurotropin on the pain of MIA-induced OA rats. To clarify the effects of repetitive dosing on OA pain, Neurotropin at doses of 100 or 200 NU/kg was orally administered to MIA-induced OA rats from day 7 to day 28. Furthermore, repetitive oral administrations of Neurotropin (200 NU/kg), loxoprofen (2 mg/kg), or meloxicam (2 mg/kg) were carried out from day 7 to day 21, and mechanical hyperalgesia and knee joint pain were measured at 24 hours after Neurotropin administration. To clarify the action mechanisms of Neurotropin, antagonists for receptor of alpha$_2$ noradrenergic, 5-HT$_{2A}$, or 5-HT$_3$ related with descending pain inhibitory pathways were intrathecally administered at 45 minutes after Neurotropin treatment, and then mechanical hyperalgesia was measured at 15 minutes after administration of antagonists.
Results: MIA at doses of 0.3, 1.0, 3.0 mg/site developed mechanical hyperalgesia and knee joint pain in a dose dependent manner. The single administration of Neurotropin (100, 200, 400 NU/kg) ameliorated mechanical hyperalgesia of OA rats in a dose dependent manner. This antinociceptive effect of Neurotropin at a dose of 200 NU/kg was reached to a peak at 1 hour and disappeared at 24 hour after Neurotropin treatment. However, the single dose of Neurotropin was not ameliorated knee joint pain of OA rats. In comparison, repetitive oral administration of Neurotropin significantly ameliorated not only mechanical hyperalgesia but also knee joint pain of OA rats even at 24 hour after Neurotropin treatment. Repetitive oral administration of loxoprofen or meloxicam was significantly ameliorated mechanical hyperalgesia and knee joint pain as well as Neurotropin treatment. However, repetitive treatment of loxoprofen or meloxicam induced gastro mucosal injury, but Neurotropin did not. The inhibitory effect of single dose of Neurotropin on mechanical hyperalgegia of OA was blocked by intrathecal injection of 30 nmol of yohimbine (alpha$_2$ noradrenaline receptor antagonist), 100 nmol of ketanserin (5-HT$_{2A}$ receptor antagonist), or 30 nmol of MDL72222 (5-HT$_3$ receptor antagonist).
Conclusions: These results indicate that the analgesic effect of Neurotropin on chronic pain of MIA-induced OA rats is comparable to NSAIDs and Neurotropin is safer than NSAIDs. The descending pain inhibitory pathway might be involved in the analgesic effect of Neurotropin on mechanical hyperalgesia of MIA-induced OA rats.

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BEHAVIOURAL ASSESSMENT OF NOCICEPTION IN OSTEOARTHRITIC RATS

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Purpose: Pain in osteoarthritis (OA) is exacerbated both by movement and by weight bearing on the affected joint. To investigate the occurrence and the time-course of pain related behaviours in an experimental model of OA, we used the knee bend, CatWalk (CW) and pin-prick tests, as well the von Frey and Randall-Selitto tests.
Methods: OA was induced by injection of mono-iodoacetate (MIA), in the left knee joint of adult male Wistar rats, at doses of 2 or 3 mg/joint. Control animals received a similar injection with saline. Paw withdrawal thresholds (PWT) to von Frey filaments and Randall-Selitto, score of knee-bend test, hind paw-print intensity (assessed using a CW apparatus and expressed as total intensity of the ipsilateral paw as a percentage of the total intensity of both hindpaws) and paw withdrawal latency (PWL) to pin-prick test were measured before the induction of OA and on various days for up to 31 days post-injection of MIA, on both sides.
Results: In every time point studied, PWT values and the hindpaw load distribution of OA rats were significantly smaller than those observed for the control group ($P<0.01$ and $P<0.05$, respectively), reflecting a mechanical allodynia. Similarly, the knee-bend score for the OA groups showed significantly higher values when compared to the control animals ($P<0.05$), indicative of the animal's allodynia and hyperalgesia. No significant difference was observed between the two OA groups in any of these tests, as well as no significant differences were observed in the Randall-Selitto and pin-prick test between the OA and the control animals.
Conclusions: We conclude that the catwalk and knee-bend test are very useful and effective methods for evaluating movement pain and disability induced by this experimental model of OA in the rat, and are better predictors of the animal allodynia and hyperalgesia than the Randall-Selitto and pin-prick tests.
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MITOCHONDRIAL PROTEIN ALTERATIONS IN HUMAN ARTICULAR CHONDROCYTES REVEALED BY A PROTEOMIC APPROACH

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Purpose: Mitochondria are involved in many cellular processes, and mitochondrial dysfunctions have been associated with apoptosis, aging and a number of pathological conditions, including osteoarthritis (OA). Mitochondrial proteins are an attractive target to study the metabolism of chondrocyte and its role in the cartilage degradation. Using a proteomic approach, we have analyzed the mitochondrial protein changes that are characteristic of OA chondrocytes, and we have identified by these means new OA-related mitochondrial proteins.
Methods: Chondrocytes were obtained from 6 OA patients undergoing joint replacement, and from 6 cartilages from autopsies without history of joint disease. Mitochondria were isolated by homogenization and differential centrifugation processes. Differential expression analysis was carried out using the differential in-gel electrophoresis technology (DIGE). Briefly, mitochondrial proteins from control and OA samples were labelled with different fluorescent dyes, mixed by pairs and co-resolved by two-dimensional gel electrophoresis using a pool of all samples as internal standard. Gel images were acquired with the Typhoon scanner, and biological variation analysis was performed using the DeCyder 6.5 software. OA-related proteins were identified by matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) or MALDI-TOF/TOF mass spectrometry. Extended statistic and clustering analysis of the data was performed with the EDA module of DeCyder software. Validation of the results was carried out by Western blotting and immunofluorescence analyses.
Results: We examined more than 1500 protein spots that were present in the six different DIGE gels. Both qualitative and quantitative changes in protein expression patterns between normal and OA chondrocyte mitochondria were studied. 36 protein spots were found to be statistically increased in OA cells compared to